European Journal of Medicinal Plants

20(3): 1-7, 2017; Article no.EJMP.33139
ISSN: 2231-0894, NLM ID: 101583475

Phytochemical Composition and Free Radicals Scavenging Activities of Methanolic Leaf Extract of *Napoleona imperialis*

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Authors' contributions

This work was carried out in collaboration between all authors. Author OEE designed the study and performed the statistical analysis. Authors FMA and OEE wrote the protocol, while author UEB wrote the first draft of the manuscript. Authors OEE and MNU managed the analyses of the study. Author EIA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2017/33139
Editor(s):
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Complete Peer review History: http://www.sciencedomain.org/review-history/20739

Original Research Article

Received 31st March 2017
Accepted 27th June 2017
Published 30th August 2017

ABSTRACT

Aims: *Napoleona imperialis* is a wild plant commonly found in Southeastern part of Nigeria used mostly for the treatment of wounds. The methanolic leaves extract of *Napoleona imperialis* was qualitatively and quantitatively analyzed for the presence of bioactive secondary metabolites and its ability to scavenge 2,2-diphenyl-1-picrylhydrazine (DPPH) radical, superoxide anion radical ($O_2^{\cdot-}$) and nitric oxide radical ($NO^{\cdot}$) was studied.
Methodology: The leaves of *N. imperialis* was air dried, pulverized and macerated in 80% methanol. Aliquots of the concentrated crude extract was used for qualitative and quantitative phytochemical screening. DPPH, superoxide (O_2^{-}) anion and nitric oxide radical scavenging

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capacity of varying concentrations of the extract was evaluated and compared with standard antioxidants; ascorbic acid, quercetin and tocopherol.
Results: The result showed the presence of saponins, flavonoids, glycosides, tannins, steroids, alkaloids and resins. Quantitative screening showed a high content of flavonoids and anthocyanins. DPPH radical scavenging potential of the extract was observed to be maximum at concentration of 1000 µg/ml similar to the effect of ascorbate. The extract also had a low superoxide (O_2^-) anion radical scavenging ability with IC_{50} of 20.23 µg/mL compared to quercetin (IC50 = 35.81 µg/mL). The $NO^{\cdot}$ scavenging capacity was concentration dependent with 500 µg/ml of the extracts scavenging most efficiently compared to α-tocopherol.
Conclusion: The leaves of *N. imperialis* has been observed to be rich in phytochemicals and have strong free radical scavenging potentials.

Keywords: Napoleona imperialis; phytochemicals; free radicals; antioxidant and oxidative stress.

1. INTRODUCTION

Today, more than 80% of the population in developing countries of the world depends on plants for their medical needs [1,2]. Traditional medicine has always been part of the cultural and religious life of African people. It is easily accessible and affordable to rural people [3]. It has been estimated that 25% of prescribed medicines today are substances derived from plants and a recent example is artemisinin obtained from *Artemisia annu*a for the treatment of malaria [3].

Napoleona imperialis is a wild plant found in south eastern Nigeria. The plant belongs to the family known as the *lecythidaceae*, along with the cannon ball tree (*Corrupita guianensis*), which grows in most regions of Nigeria. Though *Napoleona imperialis* is one of the lesser known plants, its economic importance has partially been reported by [4,5]. These include the use of the leaves for medicinal purposes and the twigs as traditional chew sticks. It has been reported that, different parts of the plant are used for different purposes in different regions; mulching and fodder (leaves and twigs); and firewood, chewing sticks and ethnomedicine (stem and root). The juice from the fruits and pods are consumed by many while the seeds are discarded. The chemical composition of the leaf, bark and roots had been documented [6].

Phytochemicals have been isolated and characterized from fruits such as grapes and apples, vegetables such as broccoli and onion, spices such as turmeric, beverages such as green tea and red wine, as well as many other sources [7]. The phytochemicals readily found in plants include; alkaloids, tannins, flavonoids, saponins, cardiac glycosides and terpenes. These flavonoids have been known to be responsible for the myriads of pharmacological or toxicological potentials of plants [8,9].

N. imperialis have been evaluated for its medicinal, pharmacological and toxicological properties to justify its usage in ethnomedicine in traditional settings. Typically, the bark and fruit pulp are chewed to alleviate pulmonary problems. Its antihypertensive and wound healing potentials have been evaluated and documented in scientific journals [10,11] while its anti-plasmodial and antimicrobial properties have also been studied [12,13]. Recent study by Etim et al. [14] has shown that the root of *N. imperialis* has antidiarrheal and antiulcerative potentials against castor oil induced diarrhea and ethanol/aspirin induced ulcer in albino Wistar rats.

However, there is scarcity of experimental information on the *In vitro* free radical and nitric oxide scavenging activity of methanol leaves extract of *Napoleona imperialis* despite its usage in traditional medicine hence the need for the present study.

2. MATERIALS AND METHODS

2.1 Plant Material and Extract Preparation

Leaves of *N. imperialis* were obtained from Mgbirichi community in Imo State and were identified at the herbarium, University of Nigeria, Nsukka. The leaves were air dried at room temperature, crushed and blended into powder. 500g of the resulting powder was subjected to extraction with 80% methanol for 48 hours. Filtrate was obtained and concentrated in a water bath at 45°C to obtain a crude extract.

2.2 Phytochemical Screening of *N. imperialis*

Qualitatively, the presence of phytochemicals such as saponins, alkaloids, tannins, flavonoids and cardiac glycosides were evaluated using the methods of Sofowora [15], Trease and Evans [16]. Resins, proteins and carbohydrate composition of the leaves were assayed using standard reagents and procedures. The methods described by Awah et al. [17], Makkar et al. [18], Kumaran and Karunakaran [19] and Giusti and Wrolstad [20] were employed in the quantitative determination of Phenolic contents, tannins, flavonoids and anthocyanins respectively.

2.3 Experimental Design

The methanol extract of leaves of *N. imperialis* was used for the study. Aliquots of the extract were used for qualitative and quantitative phytochemical screening. Serial dilutions were used to assay for its free radical scavenging potentials with a standard antioxidant as controls to compare with the extract. The extract was diluted serially to obtain different concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125 µg/mL of the extract. The free radical scavenging activities of these concentrations were investigated. The concentration of the extract that inhibited 50% of the free radicals (IC_{50}) were determined.

2.4 *In vitro* Anti-oxidant Assay

2,2-Diphenyl-1-picrylhydrazine (DPPH) free radicals scavenging activity of the extract was examined according to the method reported by Gyamfi et al. [21] with slight modifications by Awah et al. [17]. Superoxide radical ($O_2^{\cdot}$) scavenging activity of the extract was assay based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) based on the method described by Martinez et al. [22]. Assay of nitric oxide radical ($NO^{\cdot}$) scavenging potential of the extract was based on the generation of nitric oxide ($NO^{\cdot}$) from sodium nitroprusside (SNP) according to the method described by Marcocci et al. [23] and modified by Awah et al. [17].

2.5 Statistical Analysis

Data obtained from the IC_{50} determination and in vitro Anti-oxidant assay was analyzed and graphs plotted using Graph pad Prism.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Qualitative analysis of phytochemical constituents

Qualitative analysis carried out on the leaves of the plant showed the presence of important phytochemical constituents as summarized in Table 1. Tannins and saponins were the major phytochemical constituents present in the plant in a relatively high amount.

Table 1. Qualitative phytochemical constituents of the methanol extract of leaves of *N. imperialis*

Phytochemical constituent	Relative amount
Flavonoids	+
Tannins	+++
Alkaloids	+
Steroids	+
Glycoside	+
Saponins	++
Resin	+
Protein	+
Carbohydrates	+

+ = Present in trace amount; ++ = Present in moderate amount; +++ = Present in high amount; - = absent

3.1.2 Quantitative analysis of phytochemical constituents

Anthocyanins and flavonol compounds were a major class of bioactive components in the extract.

Table 2. Quantitative Phytochemical constituents of the methanol extract of leaves of *N. imperialis*

Constituent	Concentration
Total Phenol *	0.21 ± 0.02
Tannins *	0.42 ± 0.31
Total flavonol ‡	33.41 ± 0.71
Total flavonoid ‡	15.20 ± 1.17
Total anthocyanins #	237.79 ± 11.33

Data represented as Mean ± SD (n = 5)
** Expressed as mg gallic acid equivalents (GAE) / mg dry weight plant extract*
‡ Expressed as mg quercetin equivalents (RE) / g dry weight plant extract
 # Expressed as mg of cyanidin 3-glucoside equivalents per 100 g of dried plant extracts

3.1.3 Free radical scavenging activity

Extract showed significant dose-dependent DPPH radical scavenging capacity. *N. imperialis*

Table 3. Effect of extracts on DPPH radicals and superoxide anion radical

Concentrations (µg/mL)	DPPH radical scavenging activity		Superoxide anion radical ($O_2^{\cdot-}$) scavenging activity	
	Extract	Ascorbate	Extract	Quercetin
1000	89.43 ± 0.46	93.18 ± 2.41	89.65 ± 0.46	83.88 ± 3.85
500	88.67 ± 0.92	89.00 ± 0.69	90.09 ± 3.85	72.11 ± 10.78
250	82.79 ± 7.39	84.40 ± 4.84	78.65 ± 18.79	71.90 ± 4.62
125	79.19 ± 0.77	74.09 ± 3.99	74.77 ± 18.42	66.23 ± 3.17
62.5	70.04 ± 8.16	61.28 ± 2.11	67.43 ± 14.94	59.26 ± 1.69
31.25	62.20 ± 1.39	49.16 ± 9.91	66.25 ± 14.20	58.44 ± 1.58
15.625	56.54 ± 1.69	38.02 ± 12.24	45.32 ± 11.40	35.73 ± 12.48
7.8125	28.76 ± 0.62	26.74 ± 1.73	29.19 ± 1.23	27.89 ± 1.54
IC_{50}	25.35	31.26	25.35	31.26

Data represented as mean ± SEM (n = 5)

was most efficient at a concentration of 1000 µg/inhibiting 89.43 ± 0.46% of DPPH radical compared to ascorbate at same concentration. The extract also inhibited the formation of reduced NBT in a dose dependent manner as shown in Table 3.

3.1.4 Effect of extracts on nitric oxide ($NO^{\cdot}$) radical production

Nitric oxide ($NO^{\cdot}$) released from sodium nitroprusside (SNP) has a strong NO^{+} character which can alter the structure and function of many cellular components. This study showed that the phenol rich extracts in SNP solution decreased levels of nitrite, a stable oxidation product of $NO^{\cdot}$ liberated from SNP. The extracts exhibited strong NO radical scavenging activity leading to the reduction of the nitrite concentration in the assay medium, a possible protective effect against oxidative damage. The NO scavenging capacity was concentration dependent with 500 µg/ml of the extracts scavenging most efficiently compared to α-tocopherol as shown in Fig. 1.

3.2 Discussion

Phytochemicals are chemicals derived from plants since plant produce secondary metabolites in order to prevent themselves from insect attack and plant disease which in turn have some pharmacological effects in man. Phenolic content from plant extracts have been found to correlate with radical scavenging activity [24,25]. This is because polyphenolics have high redox potentials which allow them acts as reducing agents, hydrogen donors and singlet oxygen quenchers [26] Phenolic compounds; flavonoids and flavanols are known to possess good medicinal values [27]. These phytochemicals have a lot of pharmacological properties which allow them to act as reducing agents [28]. The observed presence of tannins could be of great medicinal importance since tannins serve as a good antioxidant [29]. Therefore *N. imperialis* leaves extract are good source of antioxidants, which are widely believed to be an important line of defense against oxidative stress leading to a lot of diseases like insomnia, diabetes etc. Phenolics, tannins and saponins were the major phytochemical constituents present in relatively high amount from the result as summarized in Table 1. According to results of Etim et al. [30] total phenol (0.059±0.020 mg Gallic Acid Equivalent), total flavonoid (0.615±0.008 mg Gallic Quercetin Equivalent and total flavonols(0.70±0.058 mg Quercetin Equivalent) in roots of *N. imperialis* is lower than that of the leaves.

The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search for the *in vitro* antioxidant activity of pure compounds as well as plant extracts [31,32]. DPPH radical provides an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers [33,34]. The extract maximal DPPH free radical inhibition was found to be high at a concentration of 1000 µg/ml compared to 1000 µg/ml of standard ascorbic acid as shown in Table 3, whereas the root extract showed its maximum inhibition of 88.36±4.72% at 2500 µg/m of the extract compared to 250 µg/ml of ascorbate [30]. The maximal superoxide anion inhibition was found to be a little higher than that of 1000 µg/ml quercetin. DPPH is a stable, nitrogen-centered free radical which produces violet colour in ethanol solution. It was reduced to a yellow coloured product, diphenylpicryl hydrazine, with the addition of the fractions in a concentration dependent manner. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. All the

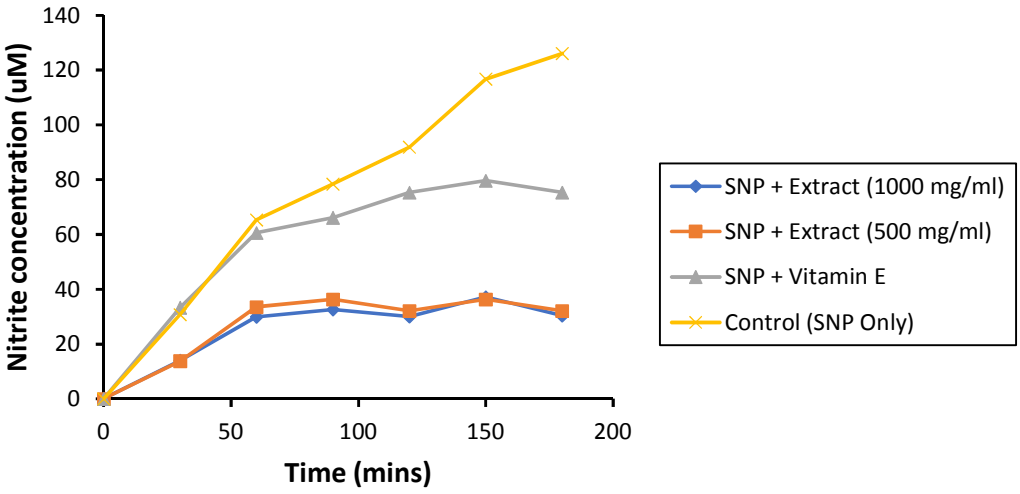

Fig. 1. Effect of extract on the accumulation of nitrite upon decomposition of SNP; Mean ± SEM (n = 5)

fractions showed significantly higher inhibition percentage (stronger hydrogen–donating ability) and positively correlated with total phenolic content. The root extract of *N. imperialis* showed a low superoxide(O2-) anion radical scavenging ability with IC50 of 1472.65 g/ml compared to Quercetin (IC50=17.018 g/ml) [30].

The IC_{50} for DPPH free radical inhibition was 25.35 µg/ml compared to 31.26 µg/ml of ascorbate while that of superoxide anion radical inhibition was 20.2 µg/ml compared to 35.81µg/ml of quercetin. The IC_{50} is the amount of extract capable of inhibiting 50% of the free radical, the lower the IC_{50} the better the potency of the extract.

In vitro inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs. Nitric oxide is a free radical which plays an important role in the pathogenesis of pain, inflammation, etc. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent 48. Nitric oxide ($NO^{\cdot}$) released from sodium nitroprusside (SNP) has a strong oxidizing character which can alter the structure and function of many cellular components. The extracts exhibited strong $NO^{\cdot}$ radical scavenging activity leading to the reduction of the nitrite concentration in the assay medium, indicating a possible protective effect against oxidative damage. The $NO^{\cdot}$ Scavenging capacity was concentration dependent with 500µg/ml of the extracts scavenging most efficiently compared to α-tocopherol. The ability of the extract to quench NO could be highly useful in preventing the formation of the very harmful peroxynitrite between superoxide anion and nitric oxide and these could go a long way to suppress oxidative stress implicated by most diseases [17].

4. CONCLUSION

The methanolic leaves extract of *Napoleona imperialis* exhibited significant antioxidant activity compared to ascorbic acid, tocopherol and quercetin possibly due to the significant presence of bioactive secondary metabolites hence its ethnomedicinal importance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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